# Diazepam

CAS #439-14-5
Sprague-Dawley rats, at 0.0, 0.04, 0.13, and 0.38% in feed
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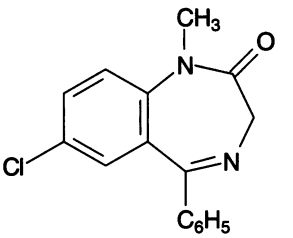


Diazepam (D), a common tranquilizer and anticonvulsant, has been in human use for many years. This RACB study in rats was performed to generate some public access information using more recent techniques on the potential for D to induce reproductive toxicity. From the dose-range-finding study (Task 1), levels of 0.04, 0.13, and 0.38% in feed were selected for the Task 2 continuous breeding phase. Based on body weight and feed consumption data, the estimated average daily diazepam doses were approximately 26, 84, and 240 mg/kg/day.

For the first generation, body weights were decreased by 13% for the middle dose males and by 12% for the high dose females, while feed consumption was reduced in both sexes of high dose animals by 5%. No adverse clinical signs were noted, nor did any any animals die during Task 2.

The number of live pups per litter was reduced by 6 and 10% for the low dose and high dose, respectively; no change from control was seen at the middle dose. Adjusted live pup weight decreased by 7% only at the high dose. The lack of an effect on pup number in the middle dose makes suspect the biological significance of the low dose change. In the absence of statistical comparisons of these small changes, these effects appeared too minor to successfully replicate in the single mating trial of a Task 3 crossover so Task 3 was not conducted.

At the end of Task 2, the last litter was reared by the dam and weaned. Although there was no change in mortality compared with controls, body weight at weaning was reduced by 9 and 14% in the middle and high dose male pups. The $F_0$ rats were killed and discarded without necropsy.

Task 4, the $F_2$ generation assessment, was conducted using the last litter from the control and high dose groups in Task 2. The body weight of the D-treated rats was approximately 8% less than the control mean, and feed consumption was reduced by approximately 7%. Although the mating indices and number of live pups per litter were unaffected by D consumption, adjusted pup weight was reduced by approximately 12% in the 0.38% D-treated rats. Although the proportion of pups born alive was significantly reduced, this is considered aberrant because the control value (100% liveborn) was slightly higher than usual, and the treated value (93% liveborn) was well within the normal control range.

After the $F_2$ litters were delivered and evaluated, the control and high dose treated $F_1$ adults were killed and necropsied. There was no change in body weight, while adjusted weights of liver and kidney were increased by 29 and 14%, respectively, while prostate weight was reduced by approximately 15%. There were no differences in sperm end points. Body weight was 10% less in D-treated females, while adjusted liver, kidney, and ovary weights were increased by 28, 12, and 21%, respectively. The estrous cycle was similar in the two groups. No exposure-related microscopic lesions were noted in the 0.38% tissues examined.

In summary, diazepam reduced adjusted pup weight in both generations and slightly reduced pup number in Task 2, in the presence of significant increases in liver and kidney weight in the $F_2$ rats.

**Summary:** NTP Reproductive Assessment by Continuous Breeding Study.

NTIS#: **PB92190578**
Chemical: **Diazepam**
CAS#: **439-14-5**
Mode of exposure: **Feed**
Species/strain: **Sprague-Dawley rats**

| $F_0$ generation — Dose concentration → | 0.04% | 0.13% | 0.38% |
|---|---|---|---|
| General toxicity | Male, female | Male, female | Male, female |
| Body weight | —, — | ↓, — | —, ↓ |
| Kidney weight[a] | •, • | •, • | •, • |
| Liver weight[a] | •, • | •, • | •, • |
| Mortality | —, — | —, — | —, — |
| Feed consumption | —, — | —, — | ↓, ↓ |
| Water consumption | •, • | •, • | •, • |
| Clinical signs | —, — | —, — | —, — |
| **Reproductive toxicity** | | | |
| x̄ litters/pair | — | — | — |
| # live pups/litter; pup wt./litter | ↓, — | —, — | ↓, ↓ |
| Cumulative days to litter | — | — | — |
| Absolute testis, epididymis weight[a] | •, • | •, • | •, • |
| Sex accessory gland weight[a] (prostate, seminal vesicle) | •, • | •, • | •, • |
| Epidid. sperm parameters (#, motility, morphology) | •, •, • | •, •, • | •, •, • |
| Estrous cycle length | • | • | • |

| Determination of affected sex (crossover) | Male | Female | Both |
|---|---|---|---|
| Dose level | • | • | • |

| $F_1$ generation — Dose concentration → | 0.04% | 0.13% | 0.38% |
|---|---|---|---|
| General toxicity | Male, female | Male, female | Male, female |
| Pup growth to weaning | —, — | ↓, — | ↓, — |
| Mortality | —, — | —, — | —, — |
| Adult body weight | •, • | •, • | —, ↓ |
| Kidney weight[a] | •, • | •, • | ↑, ↑ |
| Liver weight[a] | •, • | •, • | ↑, ↑ |
| Feed consumption | •, • | •, • | ↓, ↓ |
| Water consumption | •, • | •, • | •, • |
| Clinical signs | •, • | •, • | —, — |
| **Reproductive toxicity** | | | |
| Fertility index | • | • | — |
| # live pups/litter; pup wt./litter | •, • | •, • | —, ↓ |
| Absolute testis, epididymis weight[a] | •, • | •, • | —, — |
| Sex accessory gland weight[a] (prostate, seminal vesicle) | •, • | •, • | ↓, — |
| Epidid. sperm parameters (#, motility, morphology) | •, •, • | •, •, • | —, —, — |
| Estrous cycle length | • | • | — |

| Summary information | |
|---|---|
| Affected sex? | Unclear |
| Study confounders: | None |
| NOAEL reproductive toxicity: | <0.04% |
| NOAEL general toxicity: | 0.04% |
| $F_1$ more sensitive than $F_0$? | No |
| Postnatal toxicity: | Yes |

Legend: —, no change; •, no observation; ↑ or ↓, statistically significant change ($p<0.05$); —, —, no change in males or females. [a]Adjusted for body weight.